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Research Article

Lupeol and crotocorylifuran from Nigerian *Croton gratissimus*: Unlocking potent bioactive compounds

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Abstract

In this study, the phytochemical analysis of the stem bark and root of *Croton gratissimus* was conducted to isolate bioactive compounds. The plant materials were collected and extracted using dichloromethane (CH_2Cl_2) and methanol (MeOH). The results showed that the *C. gratissimus* root and stem bark extracts contained important phytochemicals and were active in DPPH and cytotoxicity assay. The column chromatography yielded lupeol and crotocorylifuran according to the NMR spectroscopy and literature reports. Specifically, two bioactive compounds *lupeol* and *crotocorylifuran* were isolated from the root and stem bark of *Croton gratissimus*. The compound crotocorylifuran was reported for the first time in *C. gratissimus*.

1. Introduction

The continued use of traditional medicine has produced a body of knowledge and information on the medicinal properties of herbs that can serve as the cornerstone of modern medicine [1]. Medicinal plants have long been an inexhaustible source of treatment (or cure) for African traditional healers for a variety of life-threatening diseases [2]. For centuries, people in Africa, South Asia, and Latin America have utilized *Croton* species to treat various illnesses, infections, and digestive problems [3, 4]. Various *Croton* species such as *Croton mubango* [5], *Croton megalocarpus* [6] *Croton dictyophlebodes* [7] and *Croton haumanianus*, [8, 9] have been examined in the literature for traditional medical applications.

Another *Croton* plant species of considerable medicinal importance is *Croton gratissimus* [10]. It is commonly known as lavender *Croton* or lavender fever berry. It is a shrub or tree with a corked bark that

reaches about 10 m in height in South Africa, but in other parts of Africa, it can grow to 20m high. The plant is native to central and tropical West Africa [11]. It is found in various nations from Gambia to Ghana, Guinea, Ivory Coast to Kenya, Nigeria to Uganda, Zambia, Zaïre, and Zimbabwe [12]. *Croton gratissimus* has two varieties of the plant namely, *gratissimus* var. *gratissimus* and *Croton gratissimus* var. *sub gratissimus*. The variety *gratissimus* has no hairs on the upper leaf surface, while *sub gratissimus* has stellate hairs on the upper surface [13].

Croton gratissimus is also used as a source of herbal medicine, food flavouring, and essential oils [14]. The bark sash infusion is used to treat malaria. The charred and powdered bark is used to treat bleeding gums [14]. The soup made from a decoction of its leaves is used as a wash, as well as for treating dysentery, fever, convulsions, and headache [15]. Its bark extract is used traditionally as styptic, cathartic and remedy for dropsy, indigestion, pleurisy, uterine disorder, rheumatism, and intercostal neuralgia [16]. Its young branches are dried and powdered to make perfume due to their pleasantly aromatic nature [17].

Beyond its historical and traditional uses, *Croton gratissimus* has drawn interest in scientific studies because of its outstanding bioactive properties. The extracts of *Croton gratissimus* are reported to possess strong free radical scavenging and antimicrobial activities [18-20]. The hexane, ethyl acetate, butanol and 20% aqueous methanol extracts of *Croton gratissimus var. gratissimus* showed weak antioxidant capacity and acetylcholinesterase (AChE) inhibitory effects. The methanol extract of the leaves and stem has demonstrated weak to strong activities against various bacterial strains [21].

Numerous studies have reported on the isolation and discovery of cembranolides (class of diterpenoids) [22, 23] new diterpenes [24] lupeol and spathulenol [25] as well as alkaloids, lipids, and phenolic compounds from the trichomes and laticifers in *Croton gratissimus*. The leaves and stem extracts of *Croton gratissimus* have been reported to exhibit antibacterial, [26] anti-inflammatory [27] antiprotozoal [28] antiplatelet and cytotoxic activity properties. Therefore, *Croton gratissimus* extracts can inhibit cancer cell growth, prevent oxidative hepatic injury [29] and uterine disorders [30] among others.

In this study, the antioxidant and cytotoxicity properties of the root and stem barks of *Croton gratissimus* were examined to isolate and characterize bioactive compounds. To the best of the authors' knowledge, this is the first study on isolating bioactive compounds from the root bark of *Croton gratissimus.* It is envisaged that the findings will provide novel insights into the antioxidant and cytotoxicity properties of the plant and build upon existing knowledge of the plant's significance in traditional medicine.

2. Materials and methods

2.1 Collection and identification of plant material

The stem bark and root of *C. gratissimus* were obtained in April, 2024 from Zaria (Kaduna State, Nigeria). The samples were subsequently identified by Dr. Umar Gallah from the Department of Botany, Ahmadu Bello University (Zaria, Nigeria).

2.2. Extraction of plant material

The plant materials were air-dried at room temperature for 30 days. The dried stem bark and root were pulverized using an electric miller. Next, about 500 g of the plant materials were extracted using 1.5 L of dichloromethane (CH_2Cl_2) and methanol (MeOH) sequentially. The plant materials were macerated in the solvents for 72 hours before filtration. Lastly, the extracts were then concentrated using a rotary evaporator.

2.3. Isolation and purification of compounds

A glass column of 2 - 3 cm in diameter was cleaned, dried, and then clamped upright into a stand for a retort. The base of the dried column was filled with a piece of cotton wool. The column was filled with silica gel (mesh size of 200 – 400 mm) that had been dissolved in 100% CH_2Cl_2. The column was lightly tapped to ensure consistent packing. A small amount of CH_2Cl_2 was used to dissolve about 5 g of the stem bark extract, and silica gel was used to lessen the extract's stickiness and viscosity before placing it on top of the column. The dissolved extract was added to the packed column and fractions (10 mL) were collected in vials. Preparatory thin-layer chromatography was also performed to further purify the compounds. The pure fractions were stored for further analysis using spectroscopic methods.

2.4. Characterization

GC-MS analysis was performed using an HP 6890 gas chromatograph coupled with an HP 5973 mass selective detector. Infrared spectra were recorded on a JASCO 302-A spectrophotometer (Thermo Scientific, Waltham, MA, USA). Nuclear magnetic resonance (NMR) experiments were carried out using the NMR spectrophotometer (Bruker AVANCE III NMR400 MHz). The spectra were recorded in $CDCl_3$ and referenced to the residual solvent peak at δ_H 7.26 for the ^1H NMR and at δ_C 77.23 for the ^{13}C NMR. The spectra were processed using the Bruker Topspin software. TLC was performed on Merck precoated silica gel 60 F254 aluminium foil, and spots were detected using an aqueous sulfuric acid spray reagent.

2.5. Anti-oxidant assay (DPPH free-radical scavenging activity)

Akar *et al*., 2017 were adopted with little modification to prepare Solutions of the extracts by dissolving 0.49, 0.98, 1.47 and 1.96 mg in 2 cm^3 of their respective solvents of extraction to yield the concentrations of 0.25, 0.5, 1.0 and 1.5 mg/cm^3, respectively. DPPH (39.4 mg) was dissolved in 100 cm^3 MeOH to yield 1M solution. The solution was allowed to stand for 10 minutes and the absorbance at 517 nm was measured. DPPH solution (2 cm^3) was added to 0.5 cm^3 of each of the test solution. The mixture was shaken and left to stand for 10 minutes and the absorbance at 517 nm of the solutions was measured against the control and the percentage inhibition was calculated as shown in Equation 1. The same procedure applied to butylated hydroxyl anisole (BHA) was used as standard. The standard was prepared in distilled water.

$$Inhibition = \frac{(A_{DPPH} - A_S)}{A_{DPPH}} \times 100\% \quad 1$$

The terms A_{DPPH} and A_S denote the absorbance of the neat DPPH and test solutions, respectively.

2.6 ABTS assay

The method is based on the ability of antioxidant molecules to quench the long-lived ABTS (a blue-green chromophore with characteristic absorption at 734 nm) compared with Trolox (a water-soluble vitamin E analogue). A stable stock solution of ABTS was produced by reacting a 7 mol/L aqueous solution of ABTS with 2.45 mol/L potassium persulfate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12–16 hours before attaining an absorbance of 734 nm. Next, 1 cm^3 of ABTS solution (156 μM in 100 mM phosphate buffer, pH 7.4), 1 cm^3 of nicotine amide dinucleotide (NADH) solution (468 μM in 100 mM phosphate buffer, pH 7.4) and 1 cm^3 of sample solution (0.25, 0.5 1.0 and 1.5 mg/cm^3) in their respective solvent of extraction were mixed before noting the absorbance at 734 nm. BHA was used as a standard prepared in distilled water. The percentage inhibition of the scavenging effect of superoxide anions was calculated using Equation 2.

$$Inhibition\ (\%) = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100 \quad 2$$

The term A_{blank} denotes the absorbance of the blank in the absence of a sample, whereas A_{sample} is the absorbance in the presence of the sample.

2.7 Metal chelating assay

The reaction mixture contained 0.5 cm^3 of the extracts (0.25, 0.5, 1.0 and 1.5 mg/cm^3), 1.5 cm^3 of deionized water and 0.5 cm^3 of 1M $FeCl_2$ solution. After 30 minutes, 1.0 cm^3 of 5 M ferrozine solution was added. After 10 minutes of incubation at room temperature, the absorbance at 562 nm was measured. BHA was used as a positive control. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using Equation 3:

$$Activity\ (\%) = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100 \quad 3$$

The term $A_{control}$ denotes the absorbance of the blank in the absence of the sample, whereas A_{sample} is an absorbance in the presence of the sample.

2.8 Cytotoxic assay protocol

This was performed using the brine shrimp cytotoxic test method. For the test, 70 g of brine shrimp (*Artemia salina*) eggs were added to 250 cm^3 of seawater in a beaker and kept for 48 hours for the eggs to hatch into shrimp larvae. A portion of 0.2 g of each extract was dissolved in 2 cm^3 of its various solvents of extraction. Next, 50, 5 and 1 μ/L of each solution were drawn into vials and allowed to evaporate within 24 h. Two drops of DMSO were added and made up to 2 cm^3 with distilled water corresponding to concentrations of 1000, 100 and 10 $\mu g/cm^3$ respectively. Each dosage was prepared in triplicates, including the control. Ten (10) shrimp larvae were added to each vial. The number of the surviving shrimp at each dosage and the control

was recorded after 24 hrs. Lastly, the LC_{50} was computed using Finney probit analysis software.

3. Results and discussion

3.1 Structural elucidation of isolated compounds

The extraction yielded 42.7 g of stem bark extract, whereas the root extract was 16.49 g. The subsequent sections of the paper present results and discussions on the characterisation of the bioactive compounds from the selected *Croton* plant species.

*3.1.1. Characterization of fraction CG-1 as lupeol (**1**)*

Compound **1** was isolated as a white solid from the methanol extract of the stem bark of *C. gratissimus.* The low-resolution electron impact mass spectrum (LREIMS) for compound **1** gave a molecular ion $[M]^+$ at *m/z* = 426.2 indicating a molecular formula of $C_{30}H_{50}O$. The FTIR spectrum showed broad bands for an O-H stretch at 3416 cm^{-1} and 3055 cm^{-1} for a C=C-H stretch and at 2940 cm^{-1} and 2871 cm^{-1} for C-H stretches [31]. The ^1H NMR spectrum for compound **1** showed characteristic resonances for an isopropenyl group. This is typical of a lupane-type pentacyclic triterpenoid with the two methylene proton resonances at δ_H 4.68 (d, *J* = 2.2 Hz) and δ_H 4.57 (d, *J* = 2.2 Hz) ascribable to the two H-29 protons of lupeol. In addition, the proton (1H) NMR spectrum showed an oxymethine proton resonance that occurred at δ_H 3.17 (dd, *J* = 5.7 Hz, 11.0 Hz, H-3), which was attached to a carbon resonance at δ_C 79.2 (C-3) as observed in the HSQC/DEPT spectrum. Compound **1** was therefore identified as lupeol by comparison of its spectral data with literature reports [32]. The existence of the compound has been previously isolated and reported from *Croton haumanianus*, and *Croton gratissimus*. The ^1H and the ^{13}C NMR data are given in Table 1. The specific rotation for compound **1** was determined to be [α]19 D -22.8 (*c* 0.50, CH_2Cl_2).

3.1.2. Characterization of fraction CG-2 as Crotocorylifuran (**2**)

Compound **2** was identified as crotocorylifuran with a molecular formula of $C_{22}H_{26}O_7$ using the mass spectrum. The IR spectrum revealed the presence of two carbonyl (C=O) signals at 1760 cm^{-1} and 1720 cm^{-1}. Its ^1H spectrum showed the presence of a secondary methyl group at δ_H = 1.4 (3H, d, *J* = 7.0 Hz). Two ester methyl groups (CO_2Me) were observed at 3.70 and 3.74, while olefinic protons were observed at 5.36 (1H, t, *J* = 6.0 Hz), and 6.84 (1H, m) and protons for a monosubstituted furan ring. Crotocorylifuran has

Table 1. NMR data of compound **1** lupeol compared with literature values

Position	^{13}C NMR δ ppm (100 MHz) in $CDCl_3$	^{13}C NMR (100 MHz) in $CDCl_3$ [32]	^1H NMR δ ppm (400 MHz) $CDCl_3$ (*J* in Hz) (Lit.)
1α	38.9 CH_2	39.1	1.66 m
1β			0.98 m
2α	27.7 CH_2	27.8	1.68 m
2β			1.57 m
3	79.2 CH	79.3	3.17 dd, *J* = 5.7, 11.0
4	39.1 C	39.2	-
5	55.5 CH	55.6	0.67 d
6α	18.5 CH_2	18.7	1.50 m
6β			1.39 m
7α	34.5 CH_2	34.6	1.38
7β			
8	41.0 C	41.2	-
9	50.7 CH	50.7	1.26 m
10	37.4 C	37.5	-
11α	21.1 CH_2	21.3	1.39 m
11β			1.23 m
12α	25.4 CH_2	25.5	1.66 m
12β			1.04 m
13	38.3 CH	38.4	1.64 m
14	43.0 C	43.2	-
15α	27.6 CH_2	27.8	1.68 m
15β			1.57 m
16α	35.8 CH_2	35.9	1.46 m
16β			1.36 m
17	43.2 C	43.4	-
18	48.2 CH	48.3	2.38 *J* = 5.7
19	48.5 CH	48.6	1.34 m
20	151.2 C	151.1	-
21α	30.1 CH_2	30.2	1.90 m
21β			1.19 m
22α	40.2 CH_2	40.4	1.37 m
22β			1.18 m
23	28.4 CH_3	28.2	0.97 s
24	15.6 CH_3	15.8	0.76 s
25	16.3 CH_3	16.5	0.83 s
26	16.2 CH_3	16.3	1.03 s
27	14.8 CH_3	14.9	0.94 s
28	18.2 CH_3	18.4	0.79 s
29α	109.5 CH_2	109.6	4.68 d *J* = 2.2
29β			4.57 d *J* = 2.2
30	19.5 CH_3	19.7	1.68 s

been reported from *Croton megalocarpoides* [33] and *Croton haumanianus* [34]. Using correlations in its 2D spectra enabled the assignment of the proton and carbon signals and confirmed the structure of the compound. The ^{13}C NMR data are given in Table 2.

Table 2. NMR data of compound **2** crotocorylifuran

Position	^{13}C NMR δ ppm (100 MHz) in $CDCl_3$	^{1}H NMR δ ppm (400 MHz) $CDCl_3$ (*J* in Hz)
1α	19.2	1.89 m
1β		2.62 m
2α	26.5	2.37 m
2β		2.53 m
3	140.3	6.82 dd 3.3d, 4.2d
4	136.5	-
5	46.3	-
6α	32.3	2.91 dt, 13.3m, 3.3m
6β		1.09 dt, 3.5m, 13.3m
7α	28.0	1.56 m
7β		2.43 m
8	40.2	1.57 m
9	51.4	-
10	52.0	1.73 dd, 12.9s, 2.6d
11 α	42.5	2.41 m
11 β		2.41 m
12 α	72.0	5.43 t, 8.4d
	12β	-
13	125.6	-
14	108.3	6.38 m
15	144.3	7.43 m
16	139.6	7.45 m
17	17.2	1.11 d, 6.5d
18	167.0	-
19	173.1	-
20	176.4	-

3.2. *Antioxidant assay*

The results showed (Table 3 and Fig. 1) that the *C. gratissimus* root and stem bark extracts were active in DPPH assay. The data obtained revealed that Methanol extracts of C. gratissimus significantly scavenged DPPH radicals across a range of concentrations, with an LC_{50} value of 0.025 µg/mL. This is similar to the scavenging capabilities of gallic acid (LC_{50}: 0.02 µg/mL) [35-38] and ascorbic acid (LC_{50}: 0.01 µg/mL) [39-41].

Table 3. Percentage inhibition of free radicals by the stem bark extracts and standard compounds

Assay	Concentration (mg/mL)	Inhibition (%)
Sample in DPPH	0.25	43.5
	0.5	55.2
	1	64.3
	1.5	78.4
Sample in ABTS	0.25	42.7
	0.5	53.4
	1	59.6
	1.5	68.8
Sample in Metal Chelation	0.25	42.3
	0.5	55.7
	1	69.4
	1.5	83.3
Standard BHA	0.25	47.2
	0.5	58.5
	1	78.4
	1.5	81.2
Standard compounds		
Ascorbic acid	0.25	50.7
	0.5	62.3
	1	68.1
	1.5	78.3
α-Tocopherol	0.25	45.7
	0.5	59.4
	1	63.3
	1.5	69.7

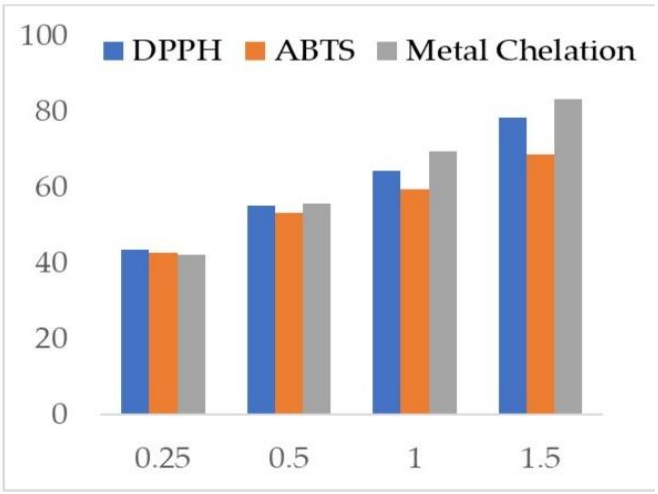

Figure 1. Percentage inhibition of the stem bark MeOH extract in DPPH, ABTS and metal chelation.

3.3 *Cytotoxic assay*

Table 4 shows the lethal concentration of the stem bark and root MeOH extracts. The LC_{50} of the stem

Table 4. Brine–shrimp lethality test result of stem bark and root MeOH crude extracts

Sample	1000 µg/mL		100 µg/mL		10 µg /mL		Control		LC_{50}
	Survivor	Dead	Survivor	Dead	Survivor	Dead	Survivor	Dead	
Stem bark	1	29	6	24	18	12	10	0	51.15
Root	2	28	8	22	16	14	10	0	71.56

bark MeOH extract was 51.15 mg/mL, which is more toxic than the root MeOH extract 71.56 mg/mL. Typically, LC_{50} values below 1000 mg/mL are considered toxic [42] therefore the two extracts are considered to be very toxic.

4. Conclusions

The study successfully isolated and characterized two bioactive compounds *lupeol* and *Crotocorylifuran* from the root and stem bark of *Croton gratissimus*. The compound *Crotocorylifuran* is reported for the first time in *C. gratissimus*. The stem bark extract was found to possess good antioxidant properties and chelating activities. However, the stem bark and root extracts exhibited cytotoxic properties. Overall, the study demonstrated that *Croton gratissimus* is a potentially practical source of bioactive compounds with future health and medicinal potentials.

Authors' contributions

Research Concept, M.S.I., A.Z.; Methodology, A.Z., M.S.I.; Formal analyses, B.M.M., Y.R., J.O.I.; Investigation, A.H.A., A.U., B.A.; Writing—original draft preparation, A.B.B.N., Writing—review and editing, A.Z.; Supervision, M.S.I.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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